TABLE 2. Diagnostic Yield of Each Examination in 25 Patients With Protein-losing Enteropathy (%)

Diagnostic Yield	EGD	Colonoscopy	FCE	VCE	Antegrade DBE	Retrograde DBE	DBE in Total
No. diagnostic	4 (16)	3 (12)	8 (32)	14 (56)	13 (52)	15 (60)	22 (88)
No. nondiagnostic	20 (80)	22 (88)	5 (20)	3 (12)	8 (32)	6 (24)	3 (12)
No. not done	1 (4)	0 (0)	12 (48)	8 (32)	4 (16)	4 (16)	0 (0)

EGD indicates esophagogastroduodenoscopy; FCE, fluoroscopic conventional enteroclysis; VCE, videocapsule endoscopy; DBE, double-balloon enteroscopy.

including biopsies. Strongyloidiasis was also diagnosed with stool samples. Twenty-four patients underwent a contrast-enhanced abdominal CT, in which abnormalities associated with PLE were detected in 17 (71%). However, as these contrast-enhanced abdominal CT findings in 17 patients had not reached the diagnosis, EGD or colonoscopy, FCE, VCE, and DBE including biopsies contributed to confirm the diagnosis and decision of management in 4 (24%), 2 (12%), 3 (18%), and 8 (47%), respectively. Although not all patients underwent all examinations, the overall diagnostic yields of EGD, colonoscopy, FCE, VCE, and DBE are presented in Table 2.

Treatment, Complications, and Prognosis

Treatment, complications, and prognoses of the 25 patients with PLE are presented in Table 3. Eight patients (32%) died of underlying disorders over the follow-up period. The median age at death was 66 (range, 32 to 73) years. There were no complications associated with DBE, VCE, and FCE.

DISCUSSION

The diagnostics of small-bowel diseases has evolved since the advent of VCE and DBE.[14,15] In this study, the detection rate of abnormalities in PLE for DBE was higher than those for FCE. To our knowledge, no comparison of DBE with other modalities for the diagnosis of PLE has been performed. Intestinal lymphangiectasia was the principal underlying disease of PLE in this study, and Aoyagi et al[16] described that FCE demonstrated clearly the characteristic radiographic findings of this disease such as smooth nodular protrusions and thickening of the mucosal folds without ulceration, compared with single-contrast study. Other characteristic findings of white plaques and white-tipped villi,[17] however, may be difficult to detect at enteroclysis and demonstrated only at enteroscopy.

TABLE 3. Treatment and Prognosis of 25 Patients With Protein-losing Enteropathy

Final Diagnosis	No.	Treatment	Complications and Prognosis
Intestinal lymphangiectasia	8		
Primary	7	7 treated on corticosteroid, but only 2 recovered	1 died from heart failure 3 mo after DBE
		3 treated on octreotide, but did not recover	1 with catheter-related thrombosis in a transient CV line treated by anticoagulant therapies
		5 treated on medium-chain triglyceride and tranexamic acid	
Secondary	1	1 recovered by chemotherapy for macroglobulinemia	
Chronic nonspecific multiple ulcers unrelated to NSAIDs	5	5 treated on iron supplementation	1 with catheter-related atrial thrombosis in a dwelling CV line removed by open heart surgery
		1 treated on albumin administration monthly	
		1 with complete small-bowel obstruction on fasting 2 W treated by balloon dilation at DBE	
		1 with partial small-bowel obstruction treated by balloon dilation at DBE	
Intestinal amyloidosis	4	1 recover on corticosteroid	2 with AA-type died from ventricular fibrillation due to cardiac amyloidosis 6 mo after DBE and from hemorrhagic shock 8 mo after DBE, respectively
		3 treated on conservative therapies	
Small-bowel tumors	3	2 treated for chemotherapy for MALT lymphoma and adult T-cell lymphoma	1 with MALT lymphoma died from multiple organ failure 17 mo after DBE, and
		1 recovered by surgical resection	1 with adult T-cell lymphoma died from multiple organ failure 5 mo after DBE.
Extragastrointestinal diseases	2	2 treated on conservative therapies	2 died from heart failure 16 and 17 mo after DBE, respectively
Strongyloidiasis	1	1 recovered on ivermectin	
Crohn's disease	1	1 treated on conservative therapies	
Small-bowel ulcers due to polyarteritis nodosa	1	1 treated on steroid pulse therapy and endoxan	1 died from multiple organ failure 30 mo after DBE

AA inicates amyloid A protein; DBE, double-balloon enteroscopy; CV line, central venous line; NSAIDs, nonsteroidal anti-inflammatory drugs; MALT, mucosa-associated lymphoid tissue.

Donzelli et al[18] reported that radiologic examination disclosed the typical anomalies of intestinal lymphangiectasia only in patients who had not responded to the dietary regimen and did not disclose lymphangiectatic plaques < 1 mm. This study also showed that the detection rate of abnormalities in intestinal lymphangiectasia for DBE (88%) was higher than that for FCE (40%). For the diagnosis of the underlying diseases of PLE with subtle lesions including intestinal lymphangiectasia and ulcers due to polyarteritis nodosa as listed in this study, enteroscopy capable of direct visualization of mucosal lesions in addition to biopsy may outperform other modalities. The second cause of PLE in this study, CNSU, is a rare entity of pathologically nonspecific multiple ulcers of the small intestine, reported chiefly in Japan, and distinct from cryptogenic multifocal ulcerous stenosing enteritis in that CNSU is characterized by long-standing PLE and iron-deficiency anemia from occult bleeding, not always complicated by small-bowel obstruction, mainly affects the ileum, lacks in vascular abnormalities, and is steroidresistant.[19–21] As CNSU has characteristic FCE images and no specific pathologic findings heretofore, FCE would be preferable to DBE for screening. VCE has the similar detection rate of abnormalities to DBE as demonstrated in this study, thereby would help to diagnose and follow up the underlying diseases of PLE painlessly, but it is contraindicated for patients with intestinal obstruction. As PLE consists of diseases complicated with strictures such as CNSU, Crohn's disease, and tumors, VCE should be performed after other modalities such as CT, FCE, and the Agile patency capsule to confirm the absence of strictures.

Even though DBE had a diagnostic yield as high as 88% alone, the preceding examinations such as EGD, colonoscopy, FCE, and VCE made diagnoses in 44% of patients with PLE as shown in the present study. In other words, after these preceding examinations, less than half of patients were newly diagnosed at DBE. Fry et al[22] described that DBE had a diagnostic value of 42% in 12 patients with clinical malabsorption of unclear origin who had undergone standard upper and lower endoscopy, CT, magnetic resonance imaging enterography, and VCE; therefore, it did not mean that DBE should be used as a primary tool in these patients. This diagnostic value is similar to that in the present study. Taken together, when we encounter a patient with PLE, we would recommend initially EGD and colonoscopy, followed by FCE and VCE after confirmation of the absence of strictures, and finally DBE with biopsy, because DBE is labor intensive, occasionally invasive, and not widely available. For follow up after treatment, VCE is recommended, if VCE has observed the lesions before treatment.

The next finding of this study was that PLE is associated with poor prognosis. Of the 114 patients with PLE after Fontan operation (n = 3029), 56 (49%) died regardless of medical, interventional, or surgical treatment. Median age at diagnosis of PLE was 11.7 years with a median time interval between Fontan operation and diagnosis of 2.7 years (range, 0.1 to 16.4 y). Results of medical treatment including digitalis, ventricular afterload reduction, steroids, antiarrhythmics, and anticoagulation were often disappointing. Surgical treatment was associated with death in 62% due to insignificant improvement in hemodynamics. Interventional procedures such as fenestrating the intra-atrial septum resulted in reducing enteric protein loss in some patients, but thromboembolic event with neurological complications or spontaneous closure of fenestrations occurred. Therefore, PLE after Fontan operation was associated with a very high mortality and morbidity rate.[23] The high rate of mortality of PLE (32%) as shown in the present study is near to that of PLE after Fontan operation, presumably due to resistance to treatment or absence of effective treatment. One patient with intestinal lymphangiectasia, 2 with amyloidosis, 2 with malignant lymphoma, 2 with extragastrointestinal diseases, and 1 with polyarteritis nodosa suffered a poor outcome, regardless of medical treatment in this study.

In conclusion, this study highlights the usefulness of DBE for the diagnosis of the underlying diseases associated with PLE. DBE has its great value for patients with PLE, but noninvasive VCE might be preferable for screening and follow up of PLE without stricture. Earlier differential diagnosis of PLE at these enteroscopy modalities may lead to a more favorable clinical outcome. Furthermore, the development of effective treatments for currently intractable diseases will be needed.

ACKNOWLEDGMENT

We thank John Cole for proofreading.

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Original Paper

Digestion

Digestion 2011;84:306–314
DOI: 10.1159/000333086

Received: May 16, 2011
Accepted: September 13, 2011
Published online: October 28, 2011

Capsule-Endoscopic Findings of Ulcerative Colitis Patients

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Key Words
Capsule endoscopy · Small intestine · Ulcerative colitis

Abstract
Background/Aims: Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by diffuse mucosal inflammation, traditionally regarded as being limited to the colorectum. Although several gastroduodenal lesions have also been reported recently in cases of UC, in general, small-bowel lesions in UC are believed to be extremely rare. The aim of this study was to examine the small bowel by capsule endoscopy in patients with UC. ***Methods:*** The study was conducted in 23 well-documented UC patients and 23 control volunteers. The frequency of small-bowel lesions, the number of small-bowel lesions per patient and the capsule endoscopy score were comparatively evaluated between the two groups. ***Results:*** Of the 23 UC patients, 13 (57%) showed small-bowel lesions, and 8 (35%) had erosions. There were significant differences in the frequency of the small-bowel lesions ($p < 0.001$) and erosions ($p = 0.009$) between the two groups. The capsule endoscopy score was correlated with the UC disease activity index ($r = 0.718$, $p < 0.001$). ***Conclusions:*** This is the first capsule-endoscopic study conducted to examine the small-bowel involvement in UC patients as compared with the healthy volunteers. It was concluded that UC, a chronic inflammatory bowel disease, can also involve the small bowel.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by diffuse mucosal inflammation, mainly of the colorectum, and its etiology and pathogenesis still remain poorly understood. UC is traditionally regarded as predominantly involving the rectum and spreading proximally, generally sparing the alimentary canal proximal to the ileocecal valve. However, gastroduodenal lesions have also been reported recently in cases of UC, such as backwash ileitis and postcolectomy pouchitis [1–6]. Furthermore, some investigators have proposed that since UC is also commonly associated with extraintestinal involvement, such as of the biliary tract, anterior chamber of the eye and synovium, it should be considered as a systemic disease and not as a localized colonic disease [7–9]. Data on the small-bowel abnormalities in UC are limited, owing to the lack of availability of an optimum tool for exploring the entire length of the

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Table 1. Characteristics of the UC patients and healthy volunteers in this study

	UC group	Control group	p value
Number of subjects	23	23	
Sex (M:F)	13:10	14:9	0.764
Mean age ± SD, years	42.9 ± 18.0	40.0 ± 15.4	0.565
Median duration of UC, months (range)	78 (1–292)		
Extent of UC (pancolitis:left-sided colitis:proctosigmoiditis)	12:7:4		
DAI			
(0–2)	6		
(3–6)	10		
(7–10)	7		
(11–12)	0		
UC treatment			
No medication	13 (includes 8 first-attack UC)		
Prednisolone	0		
Mesalazine	10		

DAI = Disease activity index according to Sutherland's criteria.

small-bowel. While some reports of postcolectomy pouchitis have been published [3–5, 10], there are no reports, until date, of frequency and nature of small-bowel involvement in unoperated UC patients. Capsule endoscopy is a newly developed tool with a high diagnostic yield for small-bowel pathologies [11, 12]. In view of the scarcity of information on the small-bowel involvement in UC, we conducted the present capsule-endoscopic study to evaluate the frequency and nature of small-bowel involvement in UC patients. The aim of this pilot study was to investigate the frequency and characteristics of small-bowel lesions in patients with UC.

Subjects and Methods

This study was a prospective, endoscopist-blinded, case-control pilot study conducted in UC patients and healthy volunteers; the study was conducted in accordance with the Declaration of Helsinki. Approval for the study was obtained from the Ethics committee of Yokohama Rosai Hospital, Yokohama, Japan. Written informed consent for participation in the study was obtained from all the UC patients and volunteers.

This study was conducted between June 2009 and December 2010 at Yokohama Rosai Hospital. UC patients and volunteers were recruited by putting up a poster at the hospital. The exclusion criteria were shown later. A total of 23 patients with well-documented UC (UC group) and 23 healthy volunteers (Control group) matched for age and sex were enrolled in this study. Matching was performed by an independent person unaware of the objective of this study. The profiles of the enrolled patients and volunteers are shown in table 1. The diagnosis of UC was confirmed in all cases using widely accepted clinical, radiologic, endoscopic and pathologic criteria [13, 14]. All patients were confirmed to be fecal culture-negative and cytomegalovirus antigenemia-negative. None of the patients or volunteers had a history of treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or aspirin within 3 months prior to the start of the study. None of the subjects had a history of chronic alcohol consumption (>20 g alcohol/day), or any history of abdominal surgery. None of the volunteers had any symptoms (e.g. diarrhea or abdominal pain), any history of use of antiulcer medications (i.e. histamine H2 receptor antagonists, proton pump inhibitors, or misoprostol), or a history of cardiovascular, respiratory or gastrointestinal diseases.

The following clinical data of the enrolled patients were collected at the time of the capsule-endoscopic examination: age, sex, disease duration, location of the colonic lesions, disease activity, and medication history. Colonoscopy was performed within 1 month of enrollment. The colitis was classified into pancolitis, left-sided colitis (defined as disease extending up to the splenic flexure) or proctosigmoiditis, according to the location of the lesions. Disease activity was determined both clinically and by colonoscopy. The clinical disease activity was graded based on the clinical features and endoscopic mucosal appearances, in accordance with the criteria for determination of the Sutherland Index (disease activity index: DAI) [15]. Two physicians independently graded the endoscopic findings and DAI at the time of enrollment. A follow-up capsule endoscopy shall be performed in previously untreated patients with first-attack UC after remission is achieved (fig. 1).

Capsule Endoscopy Procedure

All the video images were reviewed using the Pill Cam SB and SB2 capsule endoscopy system (Given Imaging Ltd., Yokneam, Israel). The capsule-endoscopic examination was performed after the patients had fasted for 12 h. Fluids and light meals were allowed 2 and 4 h, respectively, after the capsule had been swallowed. Both the patients and volunteers were free to leave the hospital, with instructions to return within the 8-hour study period,

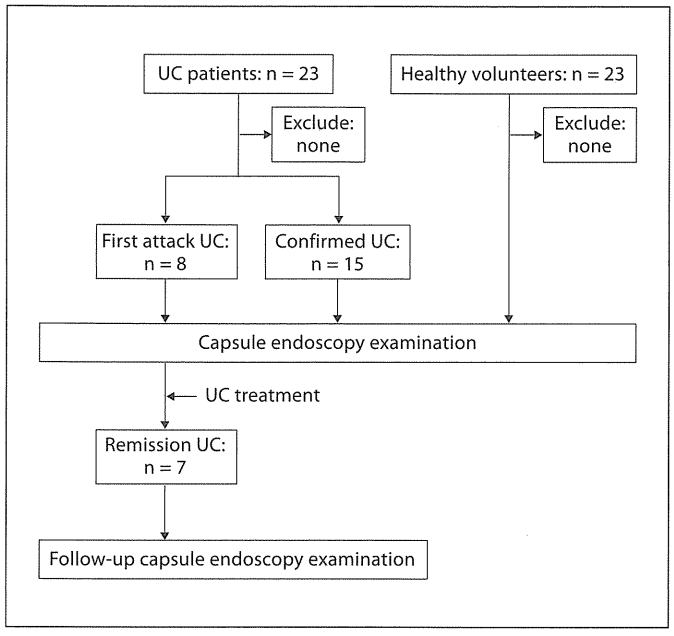

Fig. 1. Flow diagram of this study.

at the end of which the data recorder was removed. The recorded digital information was downloaded from the recorder into the computer and the images were analyzed using the proprietary RAPID software. No bowel preparation procedure, such as administration of polyethylene glycol solution or sodium phosphate, was used.

Data Analysis

Two independent investigators who were blinded to the group allocation of the patients/volunteers separately reviewed the data obtained from each of the capsule-endoscopic examinations. The small-bowel lesions were classified in severity into three types; reddened lesions, erosions, and ulcers. Reddened lesions such as reddened folds, erythema/edema and petechiae were grouped into a single category. Examples of typical reddened lesions are shown in figure 2a. Superficial white lesions with surrounding erythema were characterized as erosions. Examples of typical erosions are shown in figure 2b. White lesions within a crater and with surrounding erythema were classified as ulcers. An example of a typical ulcer is shown in figure 2c. The finding of diffuse small-bowel ulcers or multiple ulcers (>3) on capsule-endoscopic examination was considered being diagnostic of Crohn's disease, as described in a previous report [16, 17]. Then, the distribution of the small-bowel lesions was analyzed. The small bowel was divided into 3 equal segments (proximal, middle and distal) on the basis of the small-bowel transit time in each subject (see below for detailed definition of the transit time). Furthermore, we assigned the capsule endoscopy score for the small-bowel mucosal inflammatory changes in order to strengthen the validity of our results [18]. This scoring index was based on three capsule-endoscopic variables: villous appearance, ulceration, and stenosis. The mucosal inflammatory changes were assessed in tertiles, dividing the small-bowel transit time into three equal time allotments. The total score was the sum of the score for the highest tertile plus the stenosis score. The results were classified into three categories by the final numerical score: normal or clinically insignificant change (score <135), mild change (score between 135 and 790), and moderate or severe change (score ≥790).

If the judgment regarding the capsule-endoscopic findings or capsule endoscopy score assigned by the two endoscopists was different, the judgment of the preliminary endoscopist was used.

Statistical Analyses

The results were presented as mean or median (±SD or range) for quantitative data, and as frequency (percentage) for categorical data. Categorical data were analyzed using the χ^2 test or Fisher's exact test. The age, number of small-bowel erosions and ulcers, and the total number of small-bowel lesions were compared between the UC group and the control group by Student's t test. The capsule endoscopy score was also compared between the UC group and the control group, and the statistical significance of any differences was assessed by Mann-Whitney's U test. Pearson's product moment correlation coefficient (r) was calculated to explore possible correlations between the capsule endoscopy score and the DAI, capsule endoscopy score and the score for the colonic appearance of the mucosa estimated for calculation of the DAI. $p < 0.05$ was considered indicative of statistical significance.

Results

There were no examination-related complications in this study. Passage of the capsule with the stool within 2 weeks was confirmed in all the patients and volunteers (if natural passage of the capsule was not witnessed by the patient, an abdominal X-ray was obtained 14 days after the examination to confirm passage of the capsule). None of the subjects developed any adverse symptoms during the examination. In all the patients and volunteers, the capsule reached the cecum within the recording time.

Capsule-Endoscopic Findings in the Two Groups

No ulcers were seen in either group, and none of the UC patients had capsule-endoscopic findings consistent with the diagnosis of Crohn's disease.

The percentages of subjects with positive capsule-endoscopic findings (reddish lesions and erosions) in the two groups are presented in figure 3. Small-bowel lesions (reddened lesions and/or erosions) were noted in 13 of the 23 patients (57%) of the UC group, and 2 of the 23 volunteers (7%) of the control group. A statistically significant difference was observed in the frequency of small-bowel lesions between the two groups ($p < 0.001$). Erosions were seen in 8 patients (35%) of the UC group and 1 volunteer (4%) of the control group. A statistically

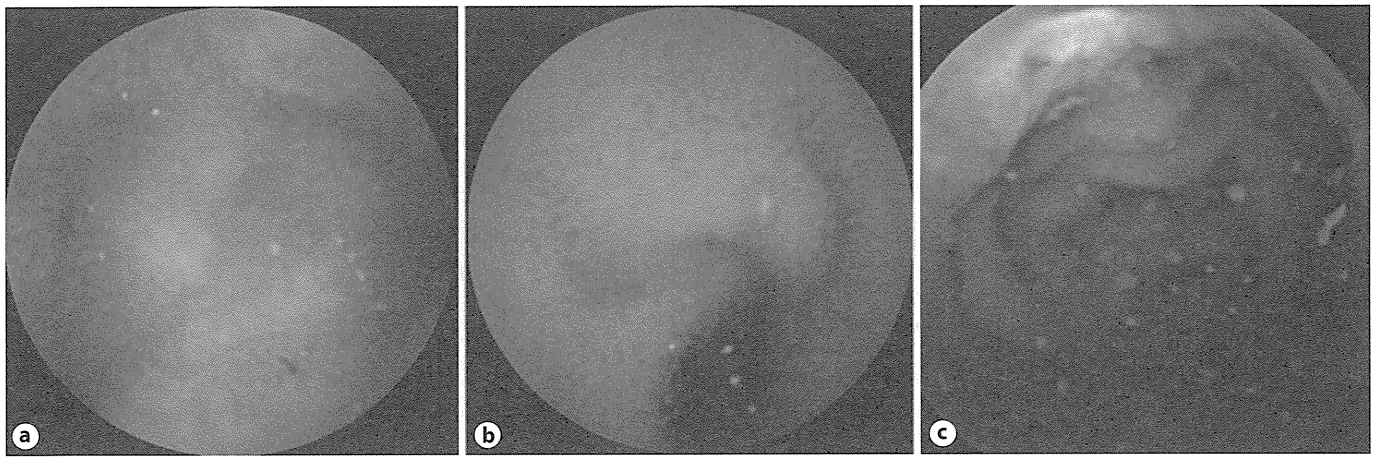

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Fig. 2. a Example of a typical reddened lesion. **b** Example of a typical erosion. **c** Example of a typical ulcer.

significant difference was observed in the frequency of small-bowel erosions between the two groups ($p = 0.009$). A representative erosion in the UC group is shown in figure 4a, b.

Comparison of the Number of Small-Bowel Lesions between the Two Groups

There was a significant difference in the total number of small-bowel lesions (reddened lesions and erosions) per subject between the UC group (mean 5.0 ± 6.4) and the control group (mean 0.1 ± 0.5; $p < 0.001$). Furthermore, there was a statistically significant difference in the number of erosions per subject between the UC group (mean 1.3 ± 2.6) and the control group (mean 0.0 ± 0.2; $p = 0.029$). The data are shown in detail in figure 5a, b.

Distribution of the Small-Bowel Lesions in the UC Patients

In the UC group, a total of 115 small-bowel lesions (85 reddened lesions and 30 erosions) were found. The distribution of the small-bowel lesions was as follows (fig. 6a, b): 28 of the 115 small-bowel lesions (24%) were located in the proximal part, 40 (35%) in the middle part, and 47 (41%) in the distal part of the small intestine. Six of the 30 erosions (20%) were located in the proximal part, 9 (30%) in the middle part, and 15 (50%) in the distal part of the small intestine. When analyzed according to the location in the small bowel, the frequency of erosions and small-bowel lesions tended to increase with progression towards the distal intestine.

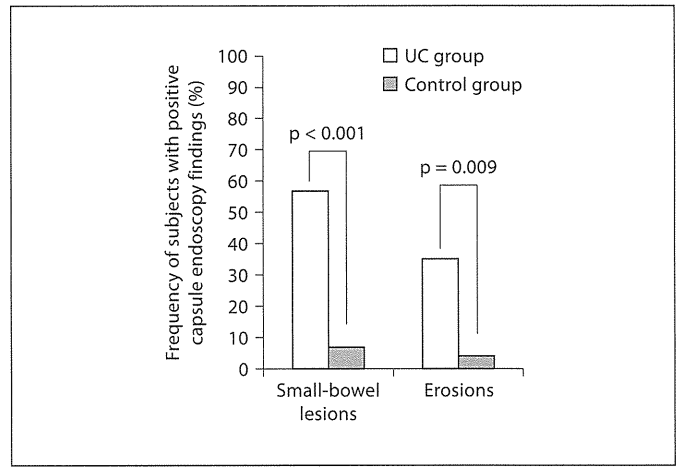

Fig. 3. Proportion of subjects with positive capsule-endoscopic findings in the two groups.

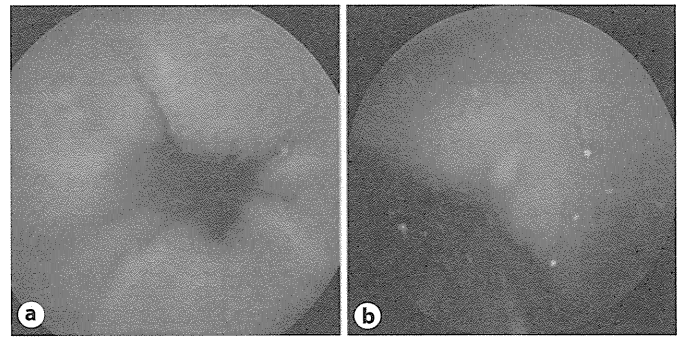

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Fig. 4. Representative erosions in the UC group.

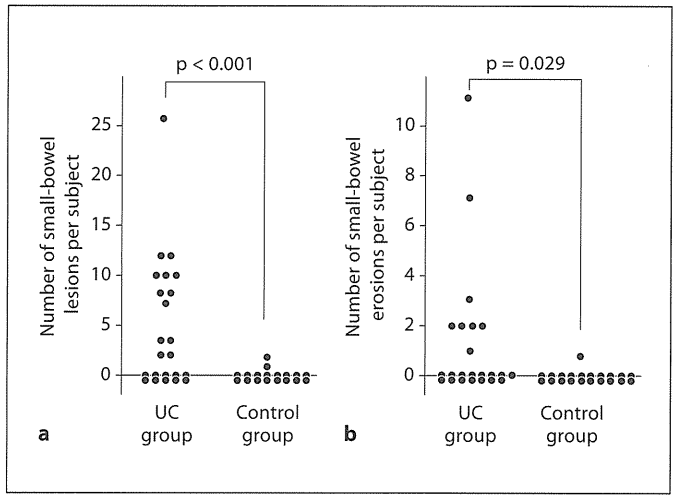


Fig. 5. a Comparison of the number of small-bowel lesions (reddened lesions and erosions). A statistically significant difference in the total number of small-bowel lesions per subject was noted between the UC group (mean 5.0 ± 6.4) and the control group (mean 0.1 ± 0.5; $p < 0.001$). **b** Comparison of the number of small-bowel erosions. A statistically significant difference in the total number of small-bowel erosions per subject was noted between the UC group (mean 1.3 ± 2.6) and the control group (mean 0.0 ± 0.2; $p = 0.029$).

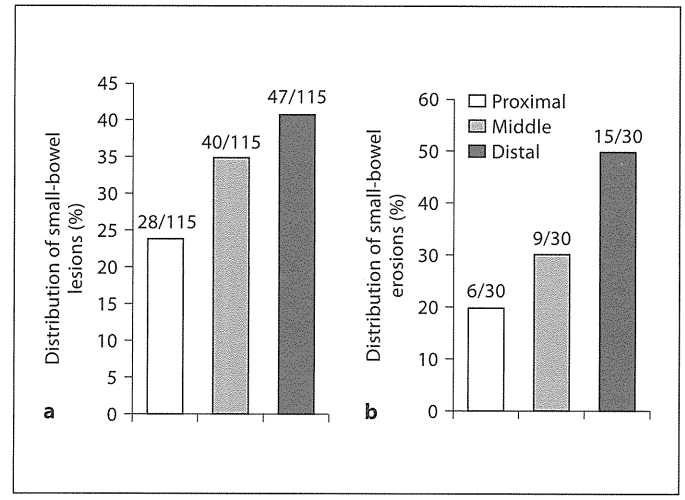


Fig. 6. a Distribution of the small-bowel lesions in the UC group; 28 of the 115 small-bowel lesions (25%) were located in the proximal part, 40 (34%) in the middle part, and 47 (41%) in the distal part of the small intestine. **b** Distribution of small-bowel erosions in the UC group; 6 of the 30 erosions (20%) were located in the proximal part, 9 (30%) in the middle part, and 15 (50%) in the distal part of the small intestine.

Comparison of the Capsule Endoscopy Score for Small-Bowel Mucosal Inflammatory Changes between the Two Groups

The capsule endoscopy score for the small-bowel mucosal inflammatory changes are shown in table 2. In the control group, the findings in all the volunteers were categorized as 'normal' or 'clinically insignificant change (score <135)'. On the other hand, in the UC group, 9 of the 23 UC patients were classified as showing 'mild change (135 ≤ score < 790)' in the small-bowel mucosa, while the remaining were categorized as normal. None of the subjects in either group in this study showed 'moderate or severe change'. There was a statistically significant difference in the proportion of subjects showing 'mild change' between the two groups (UC group 39% vs. control group 0%; $p = 0.015$). The median capsule endoscopy score in the UC group was significantly higher than that in the control group (UC group 112 (0–654) vs. control group 0 (0–112); $p < 0.001$).

Furthermore, to examine the correlation between the small-bowel inflammatory changes and the UC disease activity, we calculated the capsule endoscopy score and the DAI (fig. 7a, b). A significant correlation was observed between the capsule endoscopy score and the DAI ($r = 0.718$, $p < 0.001$), as well as between the capsule-endoscopic score and the score for the colonic appearance of the mucosa estimated for calculation of the DAI ($r = 0.554$, $p = 0.007$).

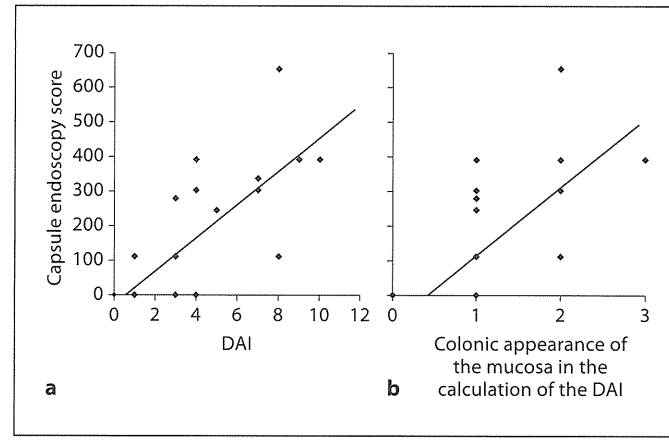
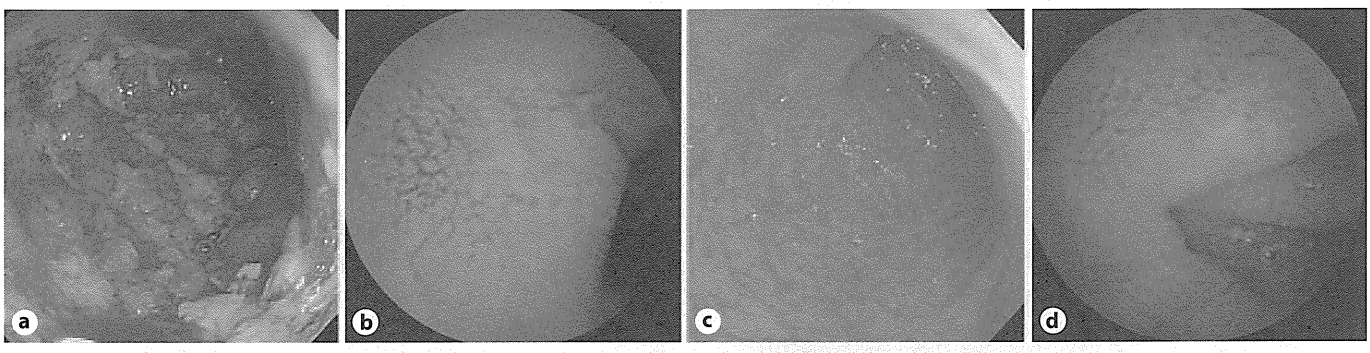


Fig. 7. a Correlation between the capsule endoscopy score and the DAI ($r = 0.718$, $p < 0.001$). **b** Correlation between the capsule endoscopy score and the score for the colonic appearance of the mucosa estimated for calculation of the DAI ($r = 0.554$, $p = 0.007$).



Color version available online

Fig. 8. a Colonoscopic findings in a first-attack UC patient before treatment. **b** Capsule endoscopy findings of the same patient before treatment. **c** Colonoscopic findings in this patient after treatment. **d** Capsule-endoscopic findings of the same patient after treatment.

Table 2. Comparison of the capsule endoscopy score for small-bowel inflammatory changes in the two groups

	UC group	Control group	p value
Median capsule endoscopy score (range)	112 (0–654)	0 (0–112)	<0.001
Categories of small-bowel mucosal inflammatory changes			
Normal or clinically insignificant change (<135)	14/23 (61%)	23/23 (100%)	0.015
Mild change (135 ≤ score < 790)	9/23 (39%)	0/23 (0%)	0.015
Moderate or severe change (≥790)	0/23 (0%)	0/23 (0%)	–

Capsule-Endoscopic Findings before and after Treatment in Previously Untreated Patients with First-Attack UC

The present study included 8 patients with first-attack UC who had no history of previous treatment for UC. Capsule-endoscopic examination revealed small-bowel lesions in 7 of these untreated UC patients. Treatment with mesalazine and/or prednisolone resulted in remission in all of the 7 patients. A follow-up capsule endoscopy was performed in these patients after remission was achieved. Improvement of the small-bowel lesions along with improvement of the patients' symptoms and colonoscopic findings was noted in these patients (fig. 8a–d). The capsule endoscopy score and DAI also improved with treatment in these patients (table 3).

Table 3. Comparison of the DAI and capsule endoscopy score measured before and after treatment in patients with first-attack UC

Patient No.	Before treatment		After treatment	
	DAI	capsule endoscopy score	DAI	capsule endoscopy score
1	4	255	2	112
4	9	393	1	112
12	8	654	2	337
15	3	112	1	0
16	8	112	1	8
18	3	280	0	0
21	4	0	1	0
23	7	337	not administered	

DAI = Disease activity index according to Sutherland's criteria.

Clinical Course of the UC Patients after the Capsule-Endoscopic Examination and Pathology of the Small-Bowel Erosions

The mean follow-up period of the UC patients after capsule endoscopy was 12.6 ± 4.6 months. In none of the subjects was the diagnosis of UC changed during the follow-up period into Crohn's disease, Behçet's disease, or other disease. Double-balloon endoscopy was performed in 2 patients who were found to have small-bowel erosions, to explore the small intestine, with biopsy of the small-bowel erosions. Cryptitis, crypt abscesses, and lymphoplasmacytic infiltration of the lamina propria mucosae were seen, while there were no granulomas, in either group of patients (data not shown).

Discussion

This is the first capsule-endoscopic study conducted to examine the small-bowel findings in UC patients as compared with those in healthy volunteers. We found a significantly higher frequency of small-bowel lesions in UC patients as compared with that in the control healthy volunteers. Similarly, the total number of small-bowel lesions and number of erosions were significantly higher in the UC group as compared with those in the control group.

It is well known that UC is often associated with extraintestinal manifestations. Previous studies have reported that extraintestinal manifestations occur in 51.5% of patients with UC [19]. UC is considered to be a multifactorial polygenic chronic inflammatory disease that predominantly affects the gastrointestinal system, with the potential also for systemic involvement, such as of the biliary tract, joints, skin and eyes [7–9]. Moreover, gastroduodenal lesions of UC have recently been reported, in addition to backwash ileitis and postcolectomy pouchitis [1–6]. It is thus not surprising that UC patients were also found to have small-bowel lesions, including reddened lesions and erosions, in this study.

Therapy with mesalazine, sulfasalazopyrine and prednisolone has been reported to be effective for the upper gastrointestinal lesions of UC, whereas UC-associated gastroduodenitis has been shown to be refractory to antisecretory therapy (i.e. histamine H2 receptor antagonists, proton pump inhibitors, etc.) [3, 5, 20]. This suggests that these gastroduodenal lesions observed in UC patients do not have a peptic basis, but represent UC-associated lesions. In this study, we performed CE before and after treatment in patients with previously untreated first-attack UC. Small-bowel lesions observed before the treatment were found to have resolved following treatment with mesalazine and/or prednisolone, similar to the case for UC-associated gastroduodenitis, which has previously been reported to be responsive to this therapy. Moreover, almost all of the patients with first-attack UC (7 of 8) in this study had small-bowel lesions. In addition, histopathologic examination of the small-bowel erosions in UC showed cryptitis, crypt abscesses, and lymphoplasmacytic infiltration of the lamina propria mucosae. The findings were similar to the pathological findings of the colonic lesions in UC, including absence of granulomas. These findings suggest that the small-bowel lesions observed by capsule endoscopy in the UC patients in this study were all related to the UC.

The etiopathogenesis of the small-bowel lesions associated with UC is unknown; however, several mechanisms have been proposed based on the results of previous clinical studies of gastrointestinal lesions associated with UC and postcolectomy pouchitis. The efficacy of antibiotics and probiotics against pouchitis suggests that bacteria may play an important role in the inflammation [21]. In addition, it has been hypothesized that molecular mimicry between epithelial protein and bacterial proteins may contribute to the pathogenesis of UC [22]. The recent advances in the understanding of the pathogenesis of inflammatory bowel disease suggest that chronic inflammation is due to the aggressive cellular immune responses to a subset of luminal bacteria [23, 24]. From these reports, it was speculated that the small-bowel lesions associated with UC could possibly develop as a result of dysregulated immune responses to bacterial antigens in genetically susceptible hosts and subsequent excessive autoimmune reactions to the small-bowel epithelium, possibly via memory T cells recruited from the small-intestinal mucosa, as demonstrated in a previous study of the colorectal lesions [25]. Intestinal bacterial flora are likely poor in the jejunum, but abundant in the ileum [26]. In our study, the number of small-bowel lesions detected tended to increase from the proximal to the distal intestine. These results suggest that intestinal bacteria may play an important role in the pathogenesis of the small-bowel lesions associated with UC.

The present study had some limitations. First, sufficient pathological proof was not obtained to indicate that the small-bowel lesions detected in the patients with UC were indeed UC-associated lesions. On the other hand, there are no specific pathological findings of UC. Cryptitis, crypt abscesses and lymphoplasmacytic infiltration of the lamina propria mucosae are seen in UC, but these are not specific diagnostic findings [27]. Furthermore, considering that almost all of previously untreated patients with first-attack UC in this study had small-bowel lesions, the small-bowel lesions observed were speculated to be UC-associated lesions. Second, although there were no patients who had findings consistent with Crohn's disease, there was the possibility that the UC patients participating in this study included patients with indeterminate colitis (IC) or inflammatory bowel disease-type unclassified (IBDU). It has been reported that a clear distinction between Crohn's disease and UC cannot be made in 10–15% of patients with inflammatory bowel disease (IBD). The term IC or IBDU is used for such patients who present with divergent clinical, endoscopic and histological features [28, 29]. Diagnosis of UC in this study was based on the typical endoscopic and histologic findings, after exclusion of other diseases (e.g. infectious colitis,

ischemic colitis, radiation colitis, collagenous colitis, microscopic colitis, and drug-induced colitis) [14, 15]; however, it has been reported that the diagnosis of IBD can sometimes change during long-term observation. In a recent report, among 18 IBDU/IC patients who underwent capsule endoscopy, 7 were diagnosed as having Crohn's disease [30]. Even if some of IBDU or IC patients were included in this study, the frequency of small-bowel lesions was still remarkably high. Third, anti-*Saccharomyces cerevisiae* mannan antibodies (ASCA) and perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) have been proposed to be clinically useful as adjunctive tools for establishing the diagnosis of IBD and for differentiating between Crohn's disease and UC [31]; however, we did not examine these markers, because ASCA is not available for use in Japan. Also, these markers are not included in the diagnostic criteria of UC in Japan, and are therefore not usually checked for in routine practice. All of the UC patients in this study showed typical clinical symptoms of UC and their endoscopic findings were also typical of UC. Moreover, patients with findings suggestive of Crohn's disease, such as gastrointestinal strictures, fistulae and abscesses, were potentially excluded. We propose to continue this investigation, and that issue is going to the next research agenda. Fourth, the UC patients enrolled in this study were almost in remission and had only mild disease activity. It is unknown whether the disease activity of UC might be related to the incidence of gastroduodenitis associated with UC and backwash ileitis [32, 33]. On the other hand, it has been reported that extraintestinal manifestations such as those related to the joints, skin and eyes are associated with UC activity in most cases [34]. In this study, the capsule endoscopy score for small-bowel mucosal inflammation was correlated with the UC-DAI. The frequency of small-bowel lesions in severe UC patients remains unknown, and further studies including severe UC patients are necessary.

In conclusion, this study suggests that small-bowel pathologies exist at a high frequency in UC patients. UC is a chronic inflammatory bowel disease that may, in addition to predominantly affecting the colon, also involve the small bowel. The severity of the small-bowel mucosal inflammatory changes in UC patients was related to the UC disease activity. Further extensive studies are required for a clearer understanding of the pathogenesis of the small-bowel lesions in UC and also of the clinical significance of UC-associated small-bowel lesions.

Disclosure Statement

None of the authors disclosed any financial relationships relevant to this publication.

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J Gastroenterol (2011) 46:894–905
DOI 10.1007/s00535-011-0410-1

ORIGINAL ARTICLE—ALIMENTARY TRACT

Efficacy of *Lactobacillus casei* treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study

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Received: 14 December 2010 / Accepted: 9 March 2011 / Published online: 10 May 2011

Abstract
Background Few studies have investigated measures to prevent small bowel injuries induced by aspirin. Our aim was to evaluate the effect of probiotic treatment on the small bowel injuries induced by chronic low-dose aspirin use.
Methods Thirty-five patients who took low-dose enteric-coated aspirin 100 mg daily (for more than 3 months) plus omeprazole 20 mg daily and were diagnosed as having unexplained iron deficiency anemia participated in this prospective randomized controlled trial. We assigned the patients to receive probiotic treatment with *Lactobacillus casei* for 3 months (*L. casei* group) or not receive the probiotic (control group). Patients underwent capsule endoscopy (CE) before and after treatment.
Results Twenty-five patients, including 13 in the *L. casei* group and 12 in the control group, underwent the full analysis. Significant decreases in the number of mucosal breaks and the CE score were observed at the 3-month evaluation in the *L. casei* group as compared with the results in the control group ($P = 0.039$). The change from the baseline in the median number of mucosal breaks in the *L. casei* group was −2, as compared with 0.5 in the control group. The change from the baseline in the median CE score in the *L. casei* group was −228 compared with −4 in the control group ($P = 0.026$).
Conclusions Co-administration of *L. casei* is effective for the treatment of aspirin-associated small bowel injury.

Keywords Low-dose aspirin · Capsule endoscopy · Small bowel · Probiotics

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Introduction

Low-dose aspirin, commonly defined as 75–325 mg daily, is widely used in the clinical setting for the prevention of primary and secondary cardiovascular and cerebrovascular thrombotic events [1–3]. However, it is well-known that the use of low-dose aspirin is also associated with a risk of serious upper gastrointestinal complications, such as peptic ulceration and bleeding [4, 5]. Until recently, attention had mainly been focused on aspirin-induced damage of the stomach and duodenum; it had remained under debate as to whether low-dose aspirin might also be injurious to the small bowel, even though 'full-dose' aspirin taken as an anti-inflammatory and analgesic medication had been well known to exert intestinal toxicity.

There has been growing interest among gastroenterologists on the adverse effects of aspirin on the small bowel, especially as new endoscopic techniques, such as capsule endoscopy (CE) and double-balloon enteroscopy, have become available for the evaluation of small bowel lesions [6, 7]. In a preliminary CE study, we demonstrated that even short-term administration of low-dose aspirin induced mild mucosal inflammation of the small bowel [8]. In addition, recent clinical studies have revealed that chronic use of low-dose aspirin causes a variety of severe lesions in the small bowel, including erosions, ulcerations and

diaphragm-like strictures [9, 10]. However, few studies have investigated measures to prevent small bowel injury induced by aspirin. The recommended treatment for small bowel injury in patients taking low-dose aspirin is withdrawal of aspirin; however, in the majority of patients, low-dose aspirin is used as an antiplatelet agent and can therefore not be discontinued on account of the increased risk of cardiovascular or cerebrovascular morbidity and mortality. Thus, novel means for the treatment of this enteropathy are urgently needed.

It has been suggested that aspirin causes gastric mucosal injury through the inhibition of cyclooxygenase (COX) and a topical irritant effect [11]. In regard to injuries of the small bowel, the same mechanisms are considered to be involved in increasing the intestinal permeability, which allows mucosal exposure to a variety of enterobacteria, with consequent bowel inflammation and injury. Inflammatory responses triggered by gram-negative bacteria have been reported to play a key role in nonsteroidal anti-inflammatory drug (NSAID)-induced enteropathy [12]. Therefore, we hypothesized that modulation of the intestinal flora might be useful as a protective measure against NSAID/aspirin-induced enteropathy.

Probiotics are living microorganisms that belong to the natural flora, and are important to the health and well-being of the host [13]. Probiotic bacteria have been demonstrated to have possible therapeutic effects against intestinal inflammation [14, 15]. Probiotic *Lactobacillus* strains have been reported to possess antimicrobial activity [16, 17]. Administration of *Lactobacillus casei* (*L. casei*) has been shown to prevent the development of experimental colitis [18]. Furthermore, recent observations also support the role of probiotics in the treatment of NSAID-induced small bowel mucosal inflammation [19, 20]. *L. casei* has been demonstrated to exhibit a preventive effect on indomethacin-induced small bowel injury in an animal experiment [19].

The aim of this study was to evaluate the effects of probiotic treatment (*L. casei*) on small bowel injury in chronic low-dose aspirin users.

Methods

Study design

This was a pilot, prospective, two-center, endoscopist-blinded, randomized, controlled study. All eligible patients from two hospitals who consented to participate in this study underwent CE at study entry (baseline CE). Eligible patients not meeting any of the exclusion criteria (see below for definitions of the exclusion criteria) were randomized at a 1:1 ratio to receive either probiotic treatment with *L. casei* (*L. casei* group) or not receive probiotic treatment (control group). Patients in the *L. casei* group received viable *L. casei* (BIOLACTIS® POWDER, Yakult Honsha, Tokyo, Japan) at doses of 45×10^8 to 63×10^9 colony-forming units (CFU) daily for 3 months; patients in the control group received no drugs. An independent clinician who was not part of the investigation conducted the allocation and block randomization according to a computer-generated schedule. Post-treatment CE was performed after 3 months of treatment. The data of patients who discontinued aspirin or probiotic use during the study period were excluded from the final analysis. This study was conducted in accordance with the Declaration of Helsinki. This two-center study was conducted with the approval of the ethics committee at both institutions (Yokohama City University Hospital and Yokohama Rosai Hospital). Written informed consent was obtained from all the patients. This trial is registered with the UMIN Clinical Trials Registry, no. UMIN000001550.

Patients

Patients taking low-dose enteric-coated aspirin 100 mg once daily (for more than 3 months) plus omeprazole 20 mg once daily, who were found to have unexplained iron deficiency anemia (decline in blood hemoglobin concentration to below 13 g/dl in men and 12 g/dl in women with iron deficiency) were eligible for inclusion in the study and for the baseline CE. All of the patients had undergone a total colonoscopy and gastroscopy prior to undergoing CE. Written informed consent for the CE procedure was obtained from all the patients. Patients were excluded from the study if they had known or suspected small bowel obstruction or stricture, swallowing disorders, an implanted pacemaker, pregnancy, history of surgical operation or radiation therapy for the abdomen, active gastrointestinal disease or inflammatory bowel disease, a history of overt gastrointestinal bleeding, positive stool cultures for any pathogens, or any serious disease of the central nervous system, liver or kidney. Patients who had taken NSAIDs, misoprostol, sulphasalazine, probiotics, prebiotics, synbiotics or antibiotics within 3 months prior to the study were also not eligible for participation in the study. The patients underwent a baseline CE examination at study entry, based on which further exclusion criteria were added, including failure to access the full length of the small bowel and the presence of small bowel lesions that could cause iron deficiency anemia, such as angioectasia and tumors.

Capsule endoscopy procedure and evaluation

All videos were reviewed using the PillCam SB and PillCam SB2 CE system (Given Imaging Ltd., Israel). CE was

performed after a 12-h fasting period. No bowel preparations, such as polyethylene glycol solution or sodium phosphate, were used.

Two independent investigators (H.E. and T.H.) who were blinded to the allocation status of the subjects to the *L. casei* or control group separately reviewed each of the CE examinations. If the two investigators reported different findings for a particular lesion, a consensus was reached through discussion. The small bowel mucosal injury was classified into mucosal breaks or reddened lesions as follows [21]: mucosal breaks were defined as lesions with central pallor and surrounding erythema; neither the depth of the ulcers nor the size of the lesions was taken into consideration; reddened lesions were defined as reddish mucosal changes such as reddened folds, denuded areas and petechiae, all grouped into a single classification. Examples of typical mucosal breaks and reddened lesions found in this study are shown in Fig. 1. The numbers of mucosal breaks and reddened lesions of the small bowel were calculated for each patient and compared between before and after treatment, to evaluate the efficacy of probiotics in aspirin-associated enteropathy.

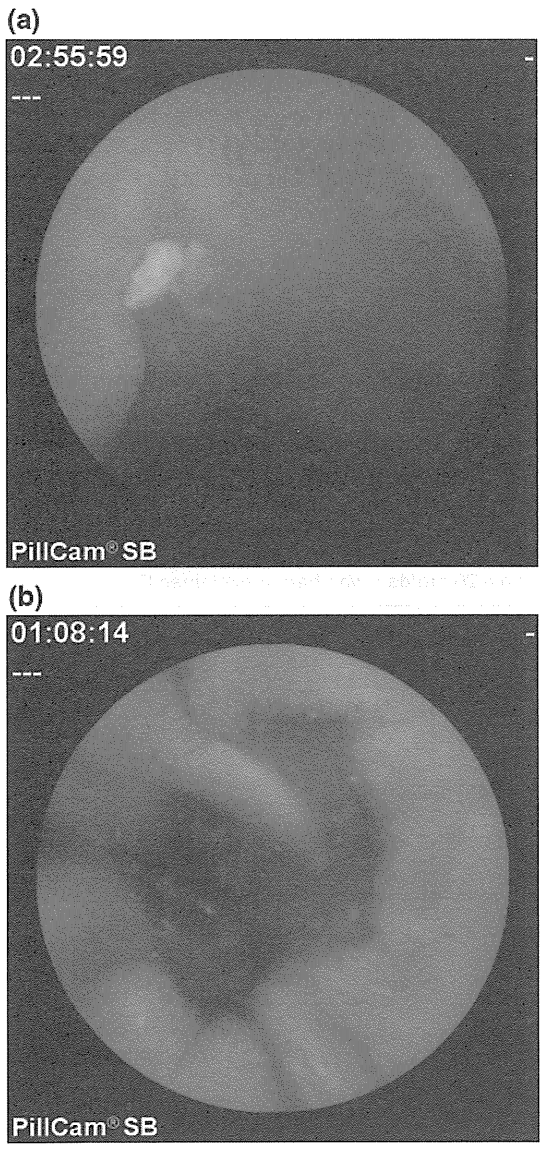

Fig. 1 Examples of a typical mucosal break (**a**) and reddened lesions (**b**) found in this study

In addition, we also determined the CE score [22] for small bowel mucosal inflammatory changes to strengthen the validity of the results. This scoring index was based on three capsule endoscopic variables: villous appearance, ulceration and stenosis. The severity of the mucosal inflammatory changes was assessed by tertiles, dividing the small bowel transit time into three equal time allotments. The total score was the sum of the highest tertile score plus the stenosis score. The results were classified into three categories based on the final numerical score: normal or clinically insignificant change (<135), mild change (between 135 and 790), and moderate or severe change (≥790). This scoring system has been shown to be useful for evaluating aspirin-associated small bowel mucosal disease activity and for objectively scoring the small bowel inflammatory disease state [23].

Laboratory studies, including a complete blood count and blood chemistry, were performed at study entry and at the end of treatment.

The primary efficacy endpoint of this study was the changes in the numbers of small bowel lesions (mucosal breaks and reddened lesions) and of the CE score from the baseline CE to the post-treatment CE performed after 3 months of treatment. The percentage of patients with at least one mucosal break was also calculated in both groups. The secondary endpoints included the change from the baseline to the post-treatment assessment of the serum hemoglobin concentration.

Safety assessment

A safety assessment was carried out based on documentation of any adverse events that occurred during the study period.

Statistical analysis

The results were presented as the mean or median (± standard deviation or range) for quantitative data and as frequency (percentage) for the categorical data. Age and hemoglobin concentration were compared by Student's *t* test. The duration of aspirin use, the number of mucosal breaks, reddened lesions and CE score were compared by the Mann-Whitney *U* test. The proportions of patients with mucosal breaks or reddened lesions were compared by Fisher's exact test. The Wilcoxon's signed rank test was used

to compare the number of mucosal breaks/reddened lesions, CE score, and hemoglobin concentration at the baseline CE and with those at the post-treatment CE in each group. The changes from the baseline in the number of mucosal breaks/reddened lesions, CE score and hemoglobin concentration after 3 months' treatment were compared between the *L. casei* and control groups by the Mann-Whitney *U* test. *P* values of <0.05 were considered to denote statistical significance.

Results

Patient characteristics

Between May 2009 and June 2010, 35 patients participated in this trial and underwent a baseline CE examination at study entry. Among the 35 patients, 29 were found to be eligible for this study; of these, 15 were randomly assigned to the *L. casei* group and 14 to the control group. Six patients were found to be ineligible based on our exclusion criteria: four patients were excluded because of the presence of small bowel angioectasia, and two because the capsule did not reach the cecum within the reading time. None of the patients developed permanent retention of the capsule and required endoscopic/surgical removal of the capsule. After the randomization, an additional three patients, comprising one from the *L. casei* group and two from the control group, were excluded from our final analysis because of the discontinuation of the aspirin treatment for medical reasons during the study period, and a further one patient from the *L. casei* group was excluded because of poor compliance with the study medication (*L. casei*). Follow-up CE was not performed in these four patients. Thus, post-treatment CE for analysis of the changes in the small bowel lesions was carried out in 13 patients of the *L. casei* group and 12 patients of the control group. A flow chart of the study is shown in Fig. 2. All the patients' clinical data were followed for at least 3 months; however, none of the patients was newly diagnosed to have Crohn's disease, Behçet's disease or intestinal tuberculosis.

The characteristics of the patients are shown in Table 1. Of the 24 patients, 7 patients, including 3 of the *L. casei* group and 4 of the control group, were receiving aspirin in combination with another anticoagulant. There were no significant differences between the two groups at the baseline CE examination with regard to the patient characteristics, the CE findings or the CE scores. At entry, the baseline median number of mucosal breaks was 3 (range 0–41), the number of reddened lesions was 9 (range 3–37), and the baseline median CE score was 340 (range 112–1518) in the *L. casei* group, with corresponding results of 2.5 (0–91), 8 (0–16) and 348 (112–2,140), respectively, in the control group. The percentage of patients with at least one mucosal break was 84.6% in the *L. casei* group and 75.0% in the control group.

Efficacy assessment

Capsule endoscopy findings after probiotic treatment

As shown in Table 2, in the *L. casei* group, the number of mucosal breaks decreased significantly from a median of

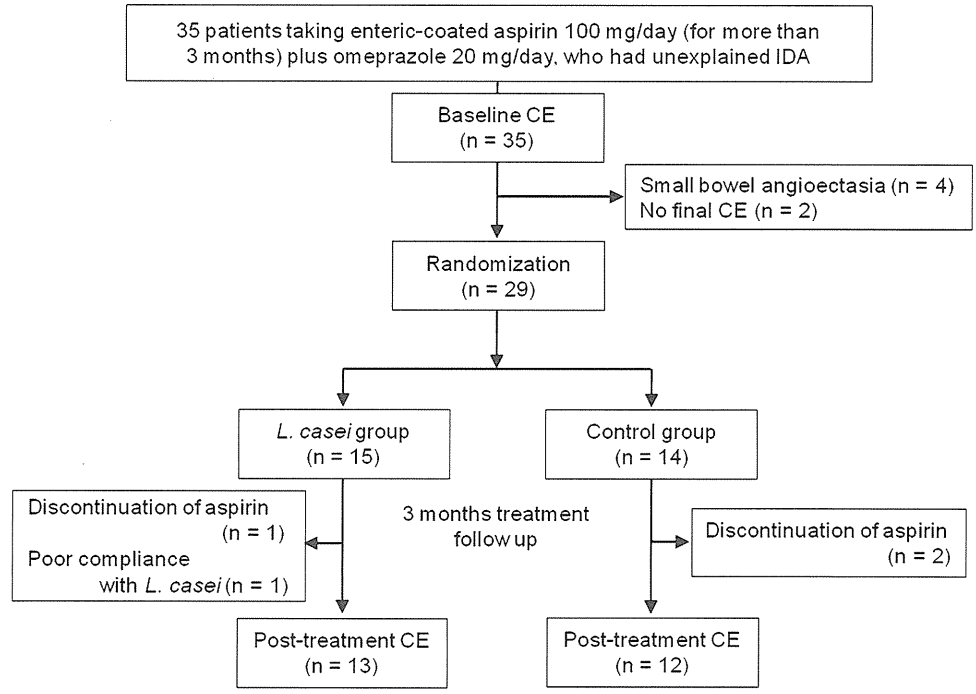

Fig. 2 Flow chart of the study patients. *CE* capsule endoscopy

Table 1 Baseline characteristics of the patients who underwent the full analysis

	L. casei ($n = 13$)	Control ($n = 12$)	*P* value
Sex (M/F)	10/3	8/4	NS
Age (years); mean ± SD	73.9 ± 8.5	70.3 ± 6.2	NS
Hemoglobin concentration (g/dl); mean ± SD	10.4 ± 2.0	10.9 ± 1.7	NS
Duration of low-dose aspirin (months); median (range)	66 (12–408)	54 (24–120)	NS
Indication for low-dose aspirin; *n* (%)			
TIA or minor ischemic stroke	5 (38.5)	4 (33.3)	NS
Prevention of recurrent myocardial infarction	4 (30.7)	3 (25.0)	NS
Chronic stable angina pectoris	3 (23.1)	5 (41.7)	NS
Valvular heart disease	1 (7.7)	0 (0)	NS
Capsule endoscopy findings			
Mucosal breaks			
Number of patients (%)	11 (84.6)	9 (75.0)	NS
Median number (range)	3 (0–41)	2.5 (0–91)	NS
Reddened lesions			
Number of patients (%)	13 (100)	11 (91.7)	NS
Median number (range)	9 (3–37)	8 (0–16)	NS
Capsule endoscopy score			
Median score (range)	340 (112–1518)	348 (112–2140)	NS
Categories of capsule endoscopy score			
Normal or clinically insignificant change (<135)	1 (7.7%)	1 (8.3%)	NS
Mild change (≥135, <790)	10 (76.9%)	10 (83.3%)	NS
Moderate or severe change (≥790)	2 (15.4%)	1 (8.3%)	NS

Table 2 Comparison of the number of patients and small bowel injuries at the baseline and post-treatment capsule endoscopy

	Baseline	Post-treatment	*P* value
L. casei group ($n = 13$)			
Mucosal breaks			
Number of patients (%)	11 (84.6)	7 (53.8)	0.202
Median number (range)	3 (0–41)	1 (0–10)	0.008
Reddened lesions			
Number of patients (%)	13 (100)	13 (100)	–
Median number (range)	9 (3–37)	4 (2–11)	0.020
Control group ($n = 12$)			
Mucosal breaks			
Number of patients (%)	9 (75.0)	10 (83.3)	>0.999
Median number (range)	3 (0–91)	3 (0–68)	0.859
Reddened lesions			
Number of patients (%)	11 (91.7)	12 (100)	>0.999
Median number (range)	8 (0–16)	7 (1–27)	0.670

three in the baseline CE to a median of one in the post-treatment CE ($P = 0.008$). In the control group, no significant difference in the median number of mucosal breaks was observed between baseline and post-treatment CE ($P = 0.859$). A decrease in the percentage of patients with at least one mucosal break was observed in response to probiotic treatment in the *L. casei* group [84.6% (11/13) in the baseline CE versus 53.8% (7/13) in the post-treatment CE]; however, the difference did not reach statistical significance ($P = 0.202$). On the other hand, in the control group, the percentage of patients with mucosal breaks increased slightly during the study period; there was no significant difference within the group between these time points ($P > 0.999$).

Reddened lesions were found in all patients, regardless of probiotic treatment, at post-treatment CE. The difference in the median number of reddened lesions before and after treatment was statistically significant ($P = 0.020$) in the *L. casei* group, but not significant ($P = 0.670$) in the control group (Table 2).

In the primary efficacy analysis, the decrease in the number of mucosal breaks from the baseline CE to the post-treatment CE was significantly greater in the *L. casei* group than that in the control group ($P = 0.039$) (Table 3). The decrease in the number of reddened lesions from the baseline CE to the post-treatment CE was also significantly greater in the *L. casei* group than that in the control group ($P = 0.005$) (Table 3).

Capsule endoscopy score for small bowel mucosal inflammatory changes

As shown in Fig. 3a, *L. casei* significantly improved the median CE scores from 340 (range 112–1518) in the

Table 3 Efficacy analysis: changes in the number of small bowel injuries, the capsule endoscopy score and the hemoglobin concentration from the baseline to the post-treatment

	L. casei ($n = 13$)	Control ($n = 12$)	*P* value
Change from baseline			
Mucosal breaks			
Mean ± SD	−5.3 ± 10.2	0.8 ± 7.9	
Median (min, max)	−2 (−38, 1)	0.5 (−23, 7)	0.039
Reddened lesions			
Mean ± SD	−7.4 ± 9.9	3.1 ± 6.3	
Median (min, max)	−5 (−34, 1)	1 (−6, 12)	0.005
Capsule endoscopy score			
Mean ± SD	−294.8 ± 288.1	−21.0 ± 261.1	
Median (min, max)	−228 (−1060, 125)	−4 (−510, 342)	0.026
Hemoglobin concentration			
Mean ± SD	1.6 ± 1.8	0.8 ± 1.5	
Median (min, max)	1.1	0.3	0.183

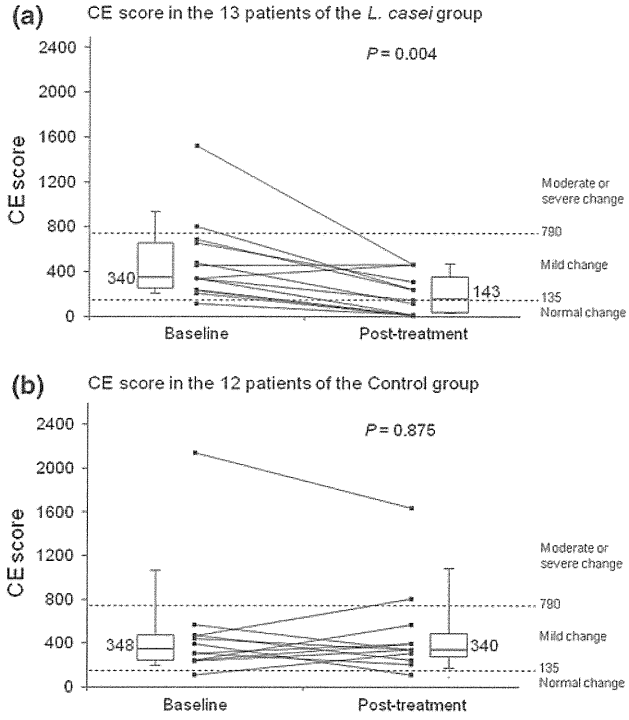

Fig. 3 Capsule endoscopy scores at the baseline capsule endoscopy and at the post-treatment capsule endoscopy in the 13 patients of the *L. casei* (**a**) and 12 patients of the control group (**b**). *CE* capsule endoscopy

baseline CE to 143 (range 8–462) in the post-treatment CE ($P = 0.004$). Both the patients who were categorized as showing moderate or severe changes (score ≥ 790) at the baseline CE in the *L. casei* group converted to mild change (135 ≤ score < 790) after treatment. Furthermore, probiotic treatment changed the category of the CE score in five of the ten patients from mild change (135 ≤ score < 790) to normal or clinically insignificant change (score < 135). In the control group, the median CE scores were comparable between baseline (348; range 112–2140) and post-treatment CE (340; range 112–1,630); this difference was not statistically significant ($P = 0.875$) (Fig. 3b). Of the ten patients who were categorized as having mild change at the baseline CE in the control group, only one converted to normal or clinically insignificant change, while in one patient, the changes were found to have deteriorated to moderate or severe change at the post-treatment CE. In one patient, normal or clinically insignificant change at the baseline CE worsened to mild change at the post-treatment CE. These results suggest the probiotic co-therapy attenuated the severity of the aspirin-associated mucosal injury.

The decrease in the CE score from the baseline CE to the post-treatment CE was significantly greater in the *L. casei* group than in the control group ($P = 0.026$) (Table 3).

Distribution of small bowel lesions

In most patients, the mucosal breaks were multifocal and were evenly distributed in the small bowel (Table 4). However, reddened lesions showed a tendency to exist in the proximal part of the small bowel (Table 4).

Table 5 compares the distribution of small bowel injuries at the baseline and post-treatment CE to evaluate the correlation between the therapeutic effect of *L. casei* and the distribution of aspirin-induced small bowel injuries. In the first tertile, significant decreases in the percentage of the patients with mucosal breaks and the number of mucosal breaks were observed at the post-treatment CE compared with the results at the baseline CE ($P = 0.047$, $P = 0.017$, respectively). A decrease in the number of

Table 4 Distribution data of small bowel injuries at the baseline capsule endoscopy

	First tertile	Second tertile	Third tertile
Baseline capsule endoscopy ($n = 25$)			
Mucosal breaks			
Number of patients (%)	14 (56.0)	12 (48.0)	18 (72.0)
Total number of lesions (mean ± SD)	82 (3.3 ± 9.3)	89 (3.6 ± 8.5)	51 (2.0 ± 2.2)
Median number (range)	1 (0–46)	0 (0–37)	1 (0–8)
Reddened lesions			
Number of patients (%)	21 (84.0)	19 (76.0)	15 (60.0)
Total number of lesions	129 (5.2 ± 5.7)	78 (3.1 ± 3.2)	55 (2.2 ± 3.0)
Median number (range)	3 (0–21)	2 (0–10)	1 (0–9)

The small bowel was divided into three parts (first, second and third tertile) on the basis of each patient's small bowel transit time

mucosal breaks in the third tertile was observed in response to probiotic treatment in the *L. casei* group; however, the difference did not reach statistical significance ($P = 0.058$).

Change in hemoglobin concentration

The hemoglobin concentration significantly increased after treatment in the *L. casei* group (median 11.1–12 g/dl; $P = 0.002$) (Fig. 4a). In the control group, on the other hand, no significant difference of the hemoglobin concentration was noted between the baseline and post-treatment CE (median 11.6–11.8 g/dl; $P = 0.196$) (Fig. 4b).

In the secondary efficacy analysis, the increase in the hemoglobin concentration from the baseline to post-treatment was greater in the *L. casei* group than in the control group; however, the difference did not reach statistical significance ($P = 0.183$) (Table 3).

Safety

No side effects or significant changes from the baseline values of any of the laboratory parameters examined were recorded in either group of patients.

Discussion

This is the first randomized controlled trial using CE performed to examine the efficacy of probiotic treatment on small bowel injury in chronic low-dose aspirin users. We found that patients treated with *L. casei* showed a significant decrease in the number of small bowel lesions associated with low-dose aspirin use. Moreover, this probiotic treatment was associated with a significant improvement in the CE scoring index. Thus, co-administration of *L. casei* decreased the incidence and severity of aspirin-associated small bowel injury.

The pathogenesis of NSAID/aspirin-induced enteropathy is likely to be multifactorial; however, the response to antibiotic treatment suggests a significant role for the enteric bacteria [24–27]. NSAID/aspirin ingestion may disrupt the homeostasis of the intestinal flora and induce overgrowth of gram-negative and anaerobic bacterial species [28]. Enterobacterial translocation into the mucosa represents the first step that sets in motion a series of events leading to gross lesion formation. In particular, gram-negative bacteria have been reported to play a key role in NSAID/aspirin-induced enteropathy [12]. The role exerted by enterobacteria in the pathogenesis of NSAID/aspirin-induced enteropathy is assumed to be similar to those of Crohn's disease [29]. Therefore, based on the previous studies showing the efficacy of probiotics against inflammatory bowel disease, we decided to use a probiotic strain for this study. Probiotic *Lactobacillus* strains, including *L. casei*, have been reported to possess antimicrobial activity [16, 17]. *Lactobacillus* strains inhibit the growth of bacterial pathogens and can even have a bactericidal effect mediated by the production of metabolites, such as lactic acid, and the resultant lowering of the pH [30]. In particular, the efficacy of *L. casei* on intestinal inflammation has been demonstrated in various studies [31, 32]. *L. casei* has been proven effective in improving murine chronic inflammatory bowel diseases by inhibiting the expression of pro-inflammatory cytokines in lamina propria mononuclear cells. The potent pro-inflammatory cytokine tumor necrosis factor α (TNF-α) seems to be one of the key factors in the pathogenesis of intestinal inflammation in both Crohn's disease and NSAID-induced enteropathy [33–35]. *L. casei* has been reported to modulate the production of TNF-α released by inflamed Crohn's disease mucosa [35]. On this basis, we decided to use a single-strain probiotic bacterium, *L. casei*. Moreover, recent studies have supported the potential therapeutic role of probiotics in small bowel inflammation induced by NSAIDs or aspirin. Watanabe et al. [19] reported that the *L. casei* strain Shirota protects against indomethacin-induced

Table 5 Comparison of the distribution of small bowel injuries at the baseline and post-treatment capsule endoscopy between the two groups

		Baseline	Post-treatment	*P* value
Mucosal breaks				
First tertile				
L. casei group ($n = 13$)				
Number of patients (%)		9 (69.2)	3 (23.1)	0.047
Total number of lesions	(mean ± SD)	27 (2.1 ± 3.4)	3 (0.2 ± 0.4)	0.017
Median number (range)		1 (0–13)	0 (0–3)	
Control group ($n = 12$)				
Number of patients (%)		5 (41.7)	7 (58.3)	0.684
Total number of lesions	(mean ± SD)	55 (4.6 ± 13.2)	42 (3.5 ± 5.6)	0.381
Median number (range)		0 (1–46)	1 (0–19)	
P value				
Number of patients		0.238	0.111	
Number of lesions		0.253	0.057	
Second tertile				
L. casei group ($n = 13$)				
Number of patients (%)		7 (53.8)	5 (38.5)	0.695
Total number of lesions	(mean ± SD)	40 (3.1 ± 6.6)	12 (0.9 ± 1.6)	0.412
Median number (range)		1 (0–24)	0 (0–5)	
Control group ($n = 12$)				
Number of patients (%)		5 (41.7)	6 (50.0)	>0.999
Total number of lesions	(mean ± SD)	49 (4.1 ± 10.5)	52 (4.3 ± 11.6)	0.795
Median number (range)		0 (0–37)	0.5 (0–41)	
P value				
Number of patients		0.695	0.695	
Number of lesions		0.724	0.532	
Third tertile				
L. casei group ($n = 13$)				
Number of patients (%)		9 (69.2)	4 (30.8)	0.115
Total number of lesions	(mean ± SD)	26 (2.0 ± 2.1)	9 (0.7 ± 1.4)	0.058
Median number (range)		2 (0–7)	0 (0–5)	
Control group ($n = 12$)				
Number of patients (%)		9 (75.0)	6 (50.0)	0.400
Total number of lesions	(mean ± SD)	25 (2.1 ± 2.5)	19 (1.6 ± 2.4)	0.387
Median number (range)		1 (0–8)	0.5 (0–8)	
P value				
Number of patients		>0.999	0.428	
Number of lesions		0.935	0.328	
Reddened lesions				
First tertile				
L. casei group ($n = 13$)				
Number of patients (%)		12 (92.3)	9 (69.2)	0.322
Total number of lesions	(mean ± SD)	80 (6.2 ± 6.4)	33 (2.5 ± 2.9)	0.058
Median number (range)		4 (0–21)	2 (0–10)	
Control group ($n = 12$)				
Number of patients (%)		9 (75.0)	11 (91.7)	0.590
Total number of lesions	(mean ± SD)	49 (4.1 ± 4.9)	69 (5.8 ± 5.4)	0.403
Median number (range)		3 (0–17)	3.5 (0–18)	